MICROBIAL COMMUNITY PATTERNS REVEAL DIFFERENCES FROM BOGS TO INTERMEDIATE FENS IN NORTH AMERICAN PEATLANDS ALONG A LATITUDINAL GRANDIENT

A Thesis
by
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Submitted to the Graduate School
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

August 2018
Department of Biology

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Abstract

Microbial Community Patterns Reveal Differences from Bogs to Intermediate Fens in North American Peatlands Along a Latitudinal Gradient

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Peatlands are unique areas of study due to their capacity to act as both carbon sinks and sources. These wetlands are estimated to hold up to one-third of the Earth's terrestrial carbon due to an unequal relationship between microbial decomposition and biological productivity, resulting in the large accumulation of peat. However, as reservoirs, peatlands are major contributors of atmospheric carbon such as carbon dioxide (CO_2) and the potent greenhouse gas, methane (CH_4). These emissions make peatlands important factors when considering the global carbon budget and the future of climate change. We analyzed the microbial community structure, as well as predictive metagenome content, in twenty North American peatlands ranging from Ontario, Canada, to North Carolina, USA. In our analysis, we described bacterial and archaeal sequences, as well as fungal sequences, with the Illumina MiSeq system using the 16S region and the internal transcribed spacer region respectively. For bacterial communities, *Proteobacteria, Acidobacteria,* and *Actinobacteria* were the dominate phyla on average

for all peatland classes. Intermediate and rich fens experienced greater diversity and taxonomic richness when compared to bogs and poor fens. Additionally, intermediate and rich fens, when combined, hosted a greater variety of candidate phyla than bogs and poor fens. Groups such as *Bacteroidetes* were observed in higher abundance in the less acidic and more nutrient rich sites, potentially aiding the higher carbon turnover rates seen in these peatlands. Supporting previous findings, archaeal sequences increased with depth for nearly all sites. Additionally, archaeal phyla *Parvachaeota* considerably increased in intermediate and rich fens. For fungal communities at phylum level, *Ascomycota* were the most abundant group on average for all peatlands classes, with the exception of rich fens where "Unknown" phyla dominated. NMDS Bray-Curtis-dissimilarity ordinations and biplots exposed pH to be the principal influence on microbial community structuring. Predictive metagenome content (PICRUSt) showed increased microbial activity, such as amino-acid and purine/pyrimidine metabolism, in relative mid-latitude peatlands from 37 to 43 degrees North, proposing a shift towards utilization of microbial biomass in these microbial communities. Ultimately, there seems to be clear differences in community composition among peatland classes, as well as distinctions in microbial metabolic activity between latitudes. These findings support the predicted rise in decomposition rates and accelerated carbon turnover, specifically for peatlands north of 37 degrees latitude.

Acknowledgments

Numerous individuals and institutions have contributed to my pursuit of a Master of Science degree. First and foremost, I would like to thank my advisor Dr. Suzanna Bräuer for taking me into her lab and for her guidance and contribution not only to my education, but to my career path as a scientist. Her passion for teaching has inspired me to become more involved as an educator. Through her knowledge and support, I was able to significantly expand my comprehension of the microbial and natural world. I am very grateful of my committee member Dr. Nathan Basiliko, for providing me the opportunities to travel, learn, and develop my skills while in Canada. I am also thankful to my committee member Dr. Matt Estep. He's mentoring and encouragement during my time here at Appalachian State University was greatly appreciated. I would also like to thank Dr. Michael Carson for his training in bioinformatics and contribution to my thesis and research.

I am appreciative to the Office of Student Research here at Appalachian State University for various funding for both research and conference travel. I am also grateful to the Basiliko lab at Laurentian University, as well as Laurentian University itself for funding multiple travel opportunities to Ontario, Canada for collaborative work.

I am forever grateful to my loving mother and father, Lynn and Jim Seward, for always encouraging and believing in me throughout my life. As well as to my sister, Elizabeth Seward, for keeping me grounded and motivated at the most difficult of times.

Dedication

I dedicate this thesis to my childhood hero and role model, my father, James Donald Seward Jr.

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Foreword

Chapter 2 of this thesis will be submitted to *Microbial Ecology*, an international peer-reviewed forum owned by *Springer* and published by *Springer US*; it has been formatted according to the style guide for that journal.

CHAPTER 1: INTRODUCTION

Peatlands are unique wetlands where decomposition is exceeded by biological productivity [1]. While peatland areas are found throughout the world, the more established and more well-studied peatlands are predominantly found in the Northern latitudes. By land area, Siberia contains the largest known peatland ecosystem. While Canada hosts the second largest, this site, the James Bay/Hudson Bay peatland complex, is arguably the largest carbon storehouse in the world. Most peatlands were formed during the retreat of the glaciers at the end of the last ice age, roughly 11,000 years ago [2]. There are however, peatlands, such as those found in the southern Appalachian Mountains, that are thought to be older than their northern counter parts, since this geographic area was not affected by glaciation. While they only take up a small percentage (3-4%) of the Earth's land surface area [2], peatlands are involved in many vital ecological and biogeographical processes [3].

Peatlands have the ability to serve as filters for many hazardous toxins and heavy metals, such as uranium [4]. More importantly, studies suggest that peatland environments hold up to one-third of the Earth's terrestrial carbon, making peatlands an important component of the planet's carbon balance [5]. Several properties contribute to peatlands' unique ability to store carbon: they are saturated, anaerobic and very low in pH and nutrient content, creating an environment that inhibits the decomposition abilities of microbes inhabiting the peat, which in turn allows for rates of biological organic production exceeding those of decomposition, and

leading to the large accumulation of carbon [6]. Peatlands host conditions that are advantageous for microbial methane production [7]. Methane has a global warming potential 23 times higher than carbon dioxide [8], which makes peatlands a crucial variable when considering future global climate change, and predicting the global carbon budget [7]. Moreover, microorganisms, such as those found in peatland habitats, have been shown to respond rapidly to environmental change [9], and this change in microbial community dynamics can significantly affect the ecosystem as a whole. Therefore, these important catalysts of peatland decomposition should be considered an essential component in climate change models.

Due to variations in climate and landforms, peatland functionality and development is not globally uniform. Peatlands are generally classified into bogs, fens (rich and poor), and marshes by differences in hydrology, water chemistry, and vegetation species [10]. Bogs, being ombrotrophic, receive water solely through precipitation, which in turn determines their low nutrient concentrations [11], in addition, bogs have lower pH, cation concentration, and alkalinity, as well as higher Sphagnum moss cover, as well as other species that can survive on relatively low nutrients, such as ericaceous shrubs, and carnivorous plants [11]. In contrast to bogs, fens receive their nutrient abundant water from geogenous aquatic sources, which stimulates higher alkalinity and higher decomposition rates [12]. These rheophilous wetlands are typically vegetated with a greater abundance of nonericaceous shrubs and graminoid species [11, 12]. Poor fens are often classified as having lower mineral composition and pH, and also having a higher percentage in Sphagnum moss cover when compared to rich fens [10, 13]. Peatland marshes, while still composing of peat and obtaining water through minerotrophic means, diverge from rich fens due to their more varied fluctuation in hydrology [13]. An increase in decomposition rates outweighing biological production is observed as you go from bog to fen

and marsh, as well as with declining latitude [13, 14]. This is most likely explained by the higher levels of microbial activity that is permitted in peatlands with higher nutrient content and pH [13], and also warmer climates [14].

An important question in the face of climate change is whether peatlands will continue to store more carbon than they release. Average global temperatures over land and sea are predicted to rise 3-5 ^{o}C and 5-7 ^{o}C respectively by the end of the century due to anthropogenic manipulation of the atmosphere [15]. In addition, the Canadian Global Climate Change Model, or CGCM1, predicts average air temperatures over northern Canada will rise by 3-4 ^{o}C by 2020, and 5-10 ^{o}C by 2050 [15]. This rise in temperature is predicted to increase the rate of decomposition of organic plant matter in peatlands , and is expected to result in the major degradation and/or drying of large northern peatlands [12, 16]. In an *in vitro* setting, peat columns from both a bog and a fen were highly sensitive to temperature increases and water table fluctuation in relation to CO_2 and CH_4 emmisions [16]. More specifically, Moore, et al. observed that CO_2 and CH_4 emmisions were 2.4 and 6.6 times higher at 23 ^{o}C than at 10 ^{o}C. Freeman, et al. (1996) provide hydrochemical data that suggest that hotter and drier conditions stimulate carbon mineralization in wetlands, as well as elevated enzyme activity as water tables rise. Precipitation in northern Canada is also expected to rise 10-20% by 2050, according to the CGCM1 [15], which would predictably trigger an increase in soil moisture. Compared to saturated columns, peat column samples with a water table level of 40 cm showed a 5-fold decrease in methane release, while carbon dioxide emissions rose 4.3 times higher on average [16]. In addition, with the predicted sea level rise, coastal flooding is expected to occur and could dramatically affect the large peatlands surrounding the Hudson Bay in Northern Canada [15]. Therefore, while drier conditions may lead to higher CO_2 emissions, wetter conditions may lead

to greater emissions of methane, a more potent greenhouse gas, and overall, predicting the moisture consistency of peatlands as global temperatures increase is valuable when considering future climate change models. Changes in vegetation cover, such as sedge encroachment, in peatlands have also been associated with increased rates in biological decomposition, which are more well supported at higher temperatures [17]. In bogs experiencing a decrease in moisture content and higher soil temperatures, shrub takeover is expected, while fens are expected to experience an increase in graminoid production and cover, and in both cases carbon and nutrient cycling may be drastically impacted [12].

One area that may hold clues as to the fate of northern peatlands as temperatures rise is the southern Appalachian Mountians. When compared to the northern peatlands, annual organic matter decomposition and net production are higher in peatlands of the southern Appalachian Mountains, and they also experience a greater rate of decomposition relative to the biological production [18]. Although peatlands of the southern Appalachian Mountains are predominately fens, it is likely that the 3-5 ^{o}C cooler temperatures during the last glacial maximum may have supported bogs in this region. Thus, it is plausible that these southern inland peatlands have already undergone an environmental change from acting as predominately carbon sinks to releasing more carbon due to a moderate rise in global temperatures and soil pH. We also predict that as global temperatures rise, as predicted by various climate models such as CGCM1, that the large high latitude peatlands will experience similar consequences.

Understanding the decomposition pathways in peatlands is crucial for assessing changes in C-storage and release in peatlands. As in most soils, fungal communities in peatlands act as primary decomposers and significantly influence carbon dynamics through degrading complex organic matter via the synthesis of extracellular enzymes [19]. Due to their hyphal growth, quick

growth rates, and ability to translocate nutrients through these hyphal networks, it is thought that fungi communities may play larger roles in the decomposition pathway of wetlands [19]. Previous studies have shown, from a taxonomic perspective, that out of 860 individual records of microfungi communities (mycota) from peatlands, 648 different species have been represented [19]. Of these, Anamorphic ascomycetes were by far the largest taxonomic groups isolated from these various peatlands [19]. Data has also shown that fungal communities in peatlands are significantly different depending on the various vegetation cover [20], since peatland bogs and fens differ in their vegetation composition, we predict to see an alteration in fungal community structure between our bogs and fens of interest. We also predict, that with this difference in fungal community structure, fens most likely host communities with higher rates of decomposition potential and are more taxonomically diverse when compared to their bog counterparts.

As with fungal communities, bacterial and archaeal groups are highly involved in the decomposition pathways, C-cycling and exchange of greenhouse gases observed in peatlands, which can vary significantly along a nutrient gradient from bogs to fens to marshes [21], making microbial metabolic activity an important aspect when predicting future CO_2 and CH_4 emissions. Along with nutrient composition, depth, pH as well as hydrology seem to act as heavy controls in regard to microbial diversity and activity [16, 22]. Peat is generally separated into oxic and anoxic zones, to which each layer hosts an array of diverse microbial communities [1]. While still moderately understudied, the interactions between aerobic and anaerobic microorganisms seem to be highly dependent on water table level and overall saturation within peatlands, which in turn can impact CO_2 and CH_4 release [16]. Due to their capacity to consume non-oxygen electron acceptors, it is thought that bacteria and archaea may be more efficiently adapted to

anaerobic conditions than their fungi counterparts [23]. Within this anaerobic layer, biological methane production (methanogenesis) ensues, which is a key aspect, as well as a terminal step in peatland carbon cycling [24]. Facilitated by methane producing archaea, or methanogens, methanogenesis utilizes substrates such as H_2/CO_2, acetate, and methanol [25]. Many of these precursors for methane assemblage are the end products of natural fermentation [24]. Herein, we aim to identify and compare these fermentative and/or hydrolytic communities and how these groups influence decomposition dynamics. In addition, we hypothesize that southern inland peatlands were once larger and deeper carbon amassing bogs, but due to a rise in global temperature, has shifted to higher water-table level and less acidic peatlands (fens) where C-release has exceeded accumulation. This microbial community comparison will give an insight into the variation in decomposition rates observed between peatland bogs and fens, as well as the potential future of high latitude North American Peatlands.

Since microorganisms will likely be the first to respond to any environmental shift and since they can catalyze changes in decompositions rates, changes in the microbial community assemblages and/or genes may serve as strong indicators or predictors of functional changes in the peat. Therefore, in the thesis work presented here, we assessed differences in microbial community assemblages between bogs and fens across a gradient of latitudes experiencing a range in mean annual air temperature (Table 1). By comparing the community structure across various peatland types, key taxonomic differences can be observed. We also aim to provide data on predictive functional gene types using PICRUSt [26]. Overall, we hypothesize that specific groups of microbes are utilizing the stored carbon and organic matter in the peat, and that an increase in temperature will allow these microbes to perform these metabolic pathways more efficiently, eventually leading to decreased carbon storage and increased nutrient content and pH

in peatlands overtime. By analyzing the fungal, archaeal, and bacterial community structure, we aim to provide evidence of accelerated decomposition rates along a gradient from bogs to fens or from Northern to Southern latitudes throughout Eastern North American peatlands from Canada to North Carolina, USA.

CHAPTER 2: Latitudinal Differences in Predictive Metagenome Content in Peatlands

Summary

Peatlands are crucial environmental variables when considering the future of climate change and global carbon budget estimates due to their capacity to act as both carbon sinks and sources. Due to an imbalance amongst plant primary production and microbial decomposition, peatlands are large accumulators of terrestrial carbon. Ironically, they are also large contributors of atmospheric carbon, particularly of the potent greenhouse gas, methane (CH_4). We sampled peat from twenty North American peatlands across nine geographically distinct locations ranging from Ontario, Canada to North Carolina, USA. In addition to latitudinal differences, these peatlands also diverged in terms of wetland classification from bog to rich fen. We described microbial community composition by means of the Illumina MiSeq system using the 16S region for bacterial and archaeal sequences and the internal transcribed region for fungal sequences. While *Proteobacteria*, *Acidobacteria*, and *Actinobacteria* were the dominant phyla on average, intermediate and rich fens hosted greater diversity and taxonomic richness, as well as an array of candidate phyla when compared to the more acidic and nutrient poor peatlands (poor fens and bogs). Additionally, groups such as *Bacteroidetes* were observed in increased abundance in the

intermediate and rich fens, potentially contributing to the more rapid carbon turnover rates experienced in these peatlands. At the phylum level, fungal communities were dominated by *Ascomycota*, except in rich fens where "Unknown" phyla took over. NMDS Bray-Curtis-dissimilarity ordinations and biplots revealed pH to be the primary influence on microbial community clustering. Predictive metagenome content (PICRUSt) showed increased microbial activity, such as purine/pyrimidine and amino-acid metabolism, in mid-latitude peatlands from 37 to 43 degrees North, suggesting a shift towards utilization of microbial biomass in these microbial communities. In all, there appears to be noticeable differences in community structure between peatland classes, as well as differences in microbial metabolic activity between latitudes. These findings are in line with a predicted increase in the decomposition and accelerated carbon turnover, particularly for peatlands north of 37 degrees latitude.

Introduction

Despite only taking up a small percentage of the Earth's land surface area (3-4%), peatlands have a large influence on global carbon cycling [3, 5], and are predicted to hold as much as one-third of the planet's terrestrial carbon [5]. In addition to storing carbon, peatlands also emit CO_2 and CH_4, depending on the water table level and degree of flooding and/or drying [27]. The low decomposition and saturated environment of the catotelm layer in peat is advantageous for microbial CH_4 production [7], which has a global warming potential 23 times higher than CO_2 under the IPCC's 100 year timeframe [8]. These emissions make peatlands a crucial variable when considering future global climate change, and predicting the global carbon budget [7].

A significant consideration in the face of climate change is whether peatlands will continue to store more carbon than they release. Average global temperatures over land and sea are predicted to rise 3-5 ^{o}C and 5-7 ^{o}C respectively by the end of the century due to anthropogenic manipulation of the atmosphere [15]. In addition, the Canadian Global Climate Change Model, or CGCM1, predicts average air temperatures over northern Canada will rise by 3-4 ^{o}C by 2020, and 5-10 ^{o}C by 2050 [15]. This rise in temperature is predicted to increase the rate of decomposition of organic plant matter in peatlands , and is expected to result in the major degradation and/or drying of large northern peatlands [12, 16]. Additionally, changes in vegetation cover, such as sedge encroachment, in peatlands have also been associated with increased rates in biological decomposition, which are more well supported at higher temperatures [17]. In an *in vitro* setting, peat columns from both a bog and a fen were highly sensitive to temperature increases and water table fluctuation in relation to CO_2 and CH_4 emmisions [16]. More specifically, Moore, et al. observed that CO_2 and CH_4 emmisions were 2.4 and 6.6 times higher at 23 ^{o}C than at 10 ^{o}C.

One area that may hold clues as to the fate of northern peatlands as temperatures rise is the southern Appalachian Mountians peatlands. When compared to the northern peatlands, annual organic matter decomposition and net production are higher in peatlands of the southern Appalachian Mountains, and they also experience a greater rate of decomposition relative to the biological production [18]. Although peatlands of the southern Appalachian Mountains are predominately fens, it is likely that the 3-5 ^{o}C cooler temperatures during the last glacial maximum may have supported bogs in this region. Thus, it is plausible that these southern inland peatlands have already undergone an environmental change from acting as predominately carbon sinks to releasing more carbon via increased microbial activity due to a moderate rise in global

temperatures and soil pH. We also predict that as global temperatures rise, as predicted by various climate models such as CGCM1, that the large high latitude peatlands may experience similar consequences.

In this study, we collected peat core samples from 20 Eastern North American peatlands across a range of 9 geographic locations. We assessed differences in microbial community (bacterial, archaeal, and fungal) assemblages and predicted functional gene types (using PICRUSt [26]) between these bogs and fens to look for differences due to latitude and wetland classification. Since peatland microbial ecology, to the best of our knowledge, is still moderately understudied, we aimed to generate a more detailed base for this microbial-wetland relationship. Our overarching goal is that insights gleaned here may facilitate future predictions of changes in peatlands in response to climate change.

Methods

Study Sites and Sample Collection

20 Eastern North American peatlands across 9 geographic locations, ranging from 36.16° to 53.69° latitudes, were chosen for this comparison study (Table 1). Peatlands were classified into 4 wetland classes (bog, poor fen, intermediate fen, and rich fen) based on the relationship between pH and Calcium content [10] (Fig. S1), hydrological input, and PI reporting. For a geographic comparison, peat samples were collected from Tennessee, USA (Ripshin); North Carolina, USA (Pineola, Sugar Hill, Tater Hill); West Virginia, USA (Cranberry Glades and Big Run); Ohio, USA (Cedar); New York, USA (McLean and Purvis Road); and Ontario, Canada (Victor Mine, Daisy Lake, Long Lake, Whitson Lake, White River, Cartier, and Mer Bleue).

Samples from each peatland were collected in triplicate cores at three depths: 10-20 cm, 30-40 cm, and 60-70 cm below the peat surface. Daisy Lake, Sugar Hill, and Tater fen lacked collection at the 60-70 cm depth, due to shallow peat profiles. Following sampling, peat was frozen (-20 °C) and shipped to the USFS lab in Houghton, MI for DNA analysis as described by Harbison et al. [28].

Chemistry and Environmental Data

Various environmental data was collected for analysis for each site and sample including *Sphagnum* and vegetation cover, along with depth to water-table, peat and water pH, core temperature, and conductivity. Average global air temperatures of peatlands from the United States were acquired from the National Oceanic and Atmospheric Administration (NOAA), while average air temperatures for the Canadian peatlands were obtained from the Government of Canada's Environment and Natural Resources website database. Total elemental analyses were collected for a suite of elements, but only chemical quantities relevant to peatlands were used in this study, including: Ca, Co, K, Mg, Na, and Ni concentrations. For each element, peat was first ashed and then fully acid digested prior to analysis on a Varian 810 ICP_MS (see Watkinson 2017) [29].

Microbial Sequencing Analysis

For community sequencing, DNA was extracted from peat samples with MoBio (now QIAGEN) Laboratories PowerSoil® DNA Isolation Kit and cleaned using the PowerClean® kit, following the manufacturers protocol with a heating step (65 °C for 30 minutes) added during the DNA extraction following bead beating. Sequences were collected on the MiSeq platform by the

Department of Energy's (DOE) Joint Genome Institute (JGI) for bacteria and archaea using the 16S region (515/806) and fungal community using the fITS9/ITS4 primer pairing. Raw sequence data was obtained from the JGI database and sequences were first quality filtered using BBMap package [30]. PANDAseq [31] was used to align forward and reverse reads, and aligned sequences were then processed with QIIME [32], and USEARCH version 8 [33] software using a 97% confidence value for OTU assignment [28, 34]. The Greengenes 2013 database [35] was used for taxonomic assignment of bacterial and archaeal assemblages, while UNITE database [36] was used for fungal identification.

Results and Discussion

Bacterial & Archaeal Community Composition

Proteobacteria were the dominant identified phylum, on average, across all sites (30.88%) followed by *Acidobateria* (19.66%) and *Actinobacteria* (6.09%; Fig. 1). Relative abundance of sequences related to *Acidobacteria* were higher in bogs and poor fens; thus, these organisms appeared to thrive in sites with greater accumulation of peat and lower turnover rates, likely reflecting the slow metabolic rates of these organisms [37, 38]. However, *Acidobacteria* were outcompeted in intermediate and rich fens as demonstrated by the rise of microbial taxonomic richness and diversity in these higher pH and more nutrient-rich peatlands (Fig. 1; Table 2). Comparatively, nutrient rich and more neutral sites (intermediate and rich fens) hosted a greater relative abundance of *Bacteroidetes*. Members of the *Bacteroidetes* phylum have high decomposition potential, and have been observed to act as primary mediators for cellulose degradation in neutral pH environments [39]. We speculate that the higher carbon turnover rates

in intermediate and rich fens is influenced, at least in part, by the increased abundance of *Bacteroidetes*.

In line with previous findings [40-42], beta-diversity, assessed via an NMDS Bray-Curtis-dissimilarity ordination, showed that pH was the strongest driver in bacterial and archaeal community clustering (Fig. 2 A), a finding supported by an NMDS biplot demonstrating that pH and Ca explained a large degree of the variance in microbial communities across a gradient from bogs to rich fens (Fig. S2). This ordination analysis (Fig. 2A) also shows overlap in microbial community assemblages within bogs and poor fens and a large separation between the microbial communities in poor vs. intermediate and rich fens. This finding is most likely due to nutrient and pH constraints on microbial communities in bogs and poor fens [40, 43]. Bacterial diversity (Shannon Diversity Index) was highest in rich fens (Table 2) with the Cedar peatland in Ohio having the highest number of detected species (4019 species) and diversity, and also the highest pH measurement. Generally, evenness increased from bog to rich fen (Table 1), although Pineola fen had the highest evenness value, indicating the lack of a dominate bacterial or archaeal group. Results corroborate previous findings demonstrating that microbial diversity in wetlands increases from bog to fen and/or to riparian environments [37, 44], most likely due to increases in pH, nutrient content, and hydrological input-s in fens and riparian wetlands.

An indicator phyla analysis was performed on all comparative peatlands by depth, demonstrating that rare and uncultivated bacterial phyla were unique to certain peatland classes (Table 3). Overwhelmingly, rich fens hosted the highest number of indicator phyla (14) between the three sample depths. Of these, candidate groups PAUC34f and GOUTA4 were most significant at lower peat depth. PAUC34f has been recently described in marine “Dead Zones” where they showed metabolic potential for complex carbohydrate compound degradation under

anaerobic conditions [45]. GOUTA4 has been observed in various environments and expresses potential for bioremediation, but its metabolic/ecological roles have yet to be fully understood [46, 47]. Compared to bogs and poor fens, intermediate and rich fens (when grouped together) experienced a large array of distinguishing indicator phyla, most of which belong to candidate divisions. The most significant, in terms of p-value, microbial groups were candidate phyla GNO2, OP11, WS3, WPS-2, GNO4, and *Gemmatimonadetes*. GNO2 has been detected, accompanied by other candidate phyla, in a sulfur-rich sands oil deposits [48]. Members of OP11are predicted to consume starch, lignin, and cellulose, as genes encoding amylopullulanase, laccase, and endoglucanase enzymes have been found in an anoxic spring [49]. These compounds are common in plants and peat, thus explaining the presence of OP11 in these nutrient-rich peatlands. WS3, now named *Latescibacteria*, has been classified as a member of the Fibrobacteres-Chlorobi-Bacteroidetes (FCM) superphylum [50]. Members of the *Latescibacteria* have been suggested to have anaerobic fermentative capabilities, with the capacity to degrade various forms of polysaccharides and glycoproteins [50]. GN02 and GN04 candidate phyla were both found in significant abundances in microbial mats in Shark Bay, Australia [51]. While still under cultivated and under studied, *Gemmatimonadetes* are thought to be as phylogenetically diverse and metabolically versatile as the more studied phyla, such as *Actinobacteria* and *Proteobacteria* [52, 53]. Although poor fens revealed no unique indicator phyla at the 10 cm depth, bogs had one indicator phylum, candidate division FBP, a group believed to share a close relationship with the recently named *Armatimonadales* phylum (previously OP10) [54]. At 60 cm, bogs hosted candidate division WPS-2. This candidate group, while still understudied, is phylogenetically related to cyanobacteria, and has been shown to tolerate polluted soils, as evidenced by the name “Wittenbug polluted soil” [55]. Overall, the large number of candidate

divisions found in this study highlights the fact that peatland microbial ecology is still fairly understudied and the fact that many peatland microbes remain uncultivated. Moreover, these candidate phyla may potentially be contributing to the increased microbial decomposition rates seen in these more nutrient rich peatlands

In line with previous studies [28], relative abundance of Archaea increased with depth from 10 to 60 cm for nearly all sites (Fig. S3). This may perhaps be explained by previous findings that archeaea can thrive under anoxic conditions and are major agents in anaerobic respiration [56, 57]. A more in-depth analysis (phylum classification) revealed that the archaeal phylum *Parvachaeota,* member of the DPANN superphylum, considerably increased in relative abundance in the intermediate and rich fens compared with poor fens and bogs. Dominance of the *Parvachaeota* was most notable in the intermediate fen, Sugar Mountain (60% of total archaeal phyla) and the rich fen, Cedar (53% of total archaeal phyla) (Fig. 3). This phylum has been described in acid mine drainage, hot springs, and marine environments [58, 59]. Genomic data suggest that members of the *Parvachaeota* could be involved in nitrogen, and carbon cycling via saccharide and protein degradation, in producing ATP through fermentation and aerobic respiration, and are likely heterotrophic since the six known carbon fixation pathways were absent from *Parvarchaeota* genomes [59]. Sequences within the phylum-level archaeal classification of "Other" also increased in average relative abundance in the intermediate and rich fens, compared to poor fens and bogs (Fig. 3). *Euryachaeota,* however, did not show a consistent trend, but demonstrated varied relative abundance values across all four peatland types. The methanogens of this phylum are of special interest in terms of carbon cycling, as they are largely responsible for CH_4 emissions observed in various wetlands [60]. Data from the literature indicate that these methanogens should be more active in higher pH sites, since CH_4

production can have a 697% increase in peat by increasing pH by just 2 units [61], perhaps due to increased bacterial activity as methanogens require bacterial end products for methanogenic substrates [60]. With the exception of Daisy Lake, *Crenarchaeota* displayed the lowest relative abundance in intermediate and rich fens. A 2006 study found that *Crenarchaeota* carry out ammonia-based chemolithoautotrophic energy metabolism in marine ecosystems [62], yet their roles in peatland nutrient cycling is still understudied.

Fungal Assemblages

Similar to bacterial and archaeal sequences, fungal groups in the selected peatlands demonstrated changes along a pH gradient along the horizontal axis in terms of beta-diversity community clustering (NMDS ordination) (Fig. 2B). This is in alignment with research carried out by Rousk et al., 2010 [41], although at least one other study has suggested that fungi communities are mainly shaped by dissolved organic carbon (DOC), ammonia, and dissolved organic nitrogen (DON), albeit in a non-peatland habitat [63]. Fungal groups, in acidic soil environments, may play a more dominate role in primary decomposition than bacteria [19]. However, bacteria have also been shown to outcompete and limit fungal growth under certain conditions such as high pH [64]. Thus, the fungal communities may be strongly influenced by bacterial and archaeal community assemblages and vice versa, perhaps explaining why these two microbial groups reflect similar community clustering trends. Fungal taxonomic analysis showed *Ascomycota* to be the dominate phylum in the bogs, poor fens, and intermediate fens whilst sequences of "Unknown" phylum were most abundant in rich fens (Fig. 4A). The high abundance of *Ascomycota* is supported by previous findings demonstrating that these fungi groups experience slow growth rates but possessed the metabolic ability to degrade complex

polymers [19]. The large relative abundance of "Unknown" fungal phyla observed in these peatlands indicates that more in-vitro isolation and sequencing should be completed to ensure a better understanding of fungal assemblages in wetland environments, as noted in other studies as well [65]. *Basidiomycota* represented the third most abundant phylum across all sites on average. However, this phylum was largely found in White River poor and White River intermediate fens, 28.73% and 24.14% respectively. *Basidiomycota*, along with *Ascomycota*, have the capacity for degradation of dissolved organic matter (DOM), such as polyphenolic compounds and cellulose [66], which may explain their abundance in peatlands. Enzymatically, *Basidiomycota* have demonstrated a higher activity of ligninolytic enzymes, as well as esterase, lipase, and arylaminidases for valine and leucine when compared to other soil fungi [67]. The relatively high abundance of *Basidiomycota* found in the White River, Ontario, Canada peatlands could be due to the fact that these fens are heavily wooded, since *Basidiomycota* have been found to be highly effective wood-decaying agents [68], and in addition many are mycorrhizal [69]. The forth most abundant phylum, *Zygomycota*, only had a noticeable abundance at site VMOE (5.92%) Victor Mine Bog, Ontario Canada. These fungi have been found to lack the proper metabolic capabilities to degrade structural plant tissues such as cellulose, tannic acid, and sphagnum polymer; potentially explaining their lower population levels in comparison to the more metabolically versatile phyla [20]. At the class level, *Leotiomycetes* (32.07%) represented the dominate fungal group on average across all sites (Fig. 4B), which is in alignment with a previous study showing the success of this taxa in boreal regions [70]. This class was most abundant in bogs (50.14%) and least copious in rich fens (8.86%) on average. While their roles in peatland environments is yet to be fully understood, *Leotiomycetes* have been found to be abundant in top layer forest soils and could be highly involved in cellulose and chitin

degradation [67]. Sequences that were "unassigned" at the class-level increased on average from bogs and poor fens (6.47%) to intermediate and rich fens (20.90%). As with bacterial communities, the high level of "unassigned" classification indicates both that the fungal communities in these wetlands are generally under cultivated, and that the more neutrally acidic and nutrient rich peatlands support higher diversity of microorganisms.

Microbial Metabolic Activity

PICRUSt [71] generation of Kyoto Encyclopedia of Genes and Genomes (KEGG) level 3 classification assigned predicted functional content (predicted metagenome content) based on the Illumina sequencing reads of the SSU rRNA gene. Forty KEGG pathways were selected (Fig. 5), where significant differences in predicted abundance were seen between sites including genes involved in environmental information processing, metabolism, genetic information processing, and cellular processing. Peatlands were organized along the x-axis in decreasing latitude, revealing that peatlands between the latitudes of 38.19° and 45.4° were predicted to have the highest number of genes associated with specific KEGG classification pathways including, but not limited to the metabolism of: methane, nitrogen, lipid, amino-acids, purine and pyrimidine. It was noted that a significant number of the genes enhanced in 38-45° North latitude peatlands were related to degradation of microbial breakdown products such as amino acids, lipids and DNA. It is speculated that these peatlands may be in a state of transition (from bogs to fens to marshes) due to global warming. In a supporting warming experiment, Tveit et al. [72] found that microbes in peat exposed to higher temperatures *in vitro* shifted their metabolism from the breakdown of plant polymers toward the breakdown of bacterial cell products [33]. In this study, as well as a similar study conducted *in situ* [73], it has also been found that increased peat

temperature leads to an increase in anoxic peat CH_4 emissions and further that the metabolic degradation of soil organic carbon (SOC) was a primary cause of this increased CH_4 production rate. Supporting these claims, a study on microbial heterotrophic activity, measured by CO_2 and CH_4 production, in everglades wetland soils found that heterotrophic utilization was highest in surface soils [74], where temperature change can more readily impact the soil processes. The data presented herein (Fig. 5), in addition to findings in the literature, predict that as temperature changes, such as increased averages in global air temperatures, certain microbial communities react and adapt swiftly, and in consequence can directly influence soil carbon cycling dynamics as well as greenhouse gas emissions.

TABLES

Table 1 Peatland geographic location; Identification; Average Annual Temperature (°C); Latitude and Longitude (decimal degrees); Elevation (m); average pH.

Location	Peatland	Average Annual Temperature (°C)	Latitude (decimal degrees)	Longitude (decimal degrees)	Elevation (m)	Average pH
Hudson Bay Lowlands Ontario, Canada	Victor Mine (VICM)	-0.56	52.720936	-83.940921	88	21:36.0
Ontario, Canada	White River Poor Fen	1.67	48.35327	-85.338231	467	4.04
Hudson Bay Lowlands Ontario, Canada	Victor Mine (VMOE Bog)	-0.56	53.692992	-83.944603	91	4.066
Ontario, Canada	Mer Bleue	4	45.41	-75.48	69	4.04
Sudbury gradient, Ontario, Canada	Cartier	4	46.39762	-81.31226	423	4.01
New York, USA	McLean	8.14	42.548812	-76.266274	341	4.09
New York, USA	Purvis Road/Dryden Bog	8.14	42.447156	-76.258488	372	4.33
West Virginia, USA	Cranberry Glades	10.16	38.1995	-80.274	1026	4.07
West Virginia, USA	Big Run	9.69	39.116859	-79.581104	981	4.67
Sudbury gradient, Ontario, Canada	Long Lake	3.5	46.37041	-81.0664	286	5.3
Sudbury gradient, Ontario, Canada	Daisy Lake	3.5	46.45491	-80.88248	249	4.8
Sudbury gradient, Ontario, Canada	Whitson Lake	3.5	46.35401	-80.59496	299	5.8
Western North Carolina	Pineola	10.14	36.023257	-81.898254	1066	5.6
Western North Carolina	Sugar	10.14	36.083455	-81.893584	1229	5.12
Western North Carolina	Tater Hill	10.14	36.283657	-81.715134	1258	5.96
Tennessee, USA	Ripshin	13.4	36.1662	-82.1512	1085	5.66
Ontario, Canada	White River Intermediate Fen	1.67	48.351015	-85.356497	485	5.37
Hudson Bay Lowlands Ontario, Canada	Victor Mine (VMOE Fen)	-0.56	52.693194	-83.944603	88	6.54
Ohio, USA	Cedar	10.5	40.0588056	-83.79438889	295	7.75

Table 2 Diversity analysis of peatlands of interest depicting total microbial species count, alpha diversity (Shannon index), and evenness. Degree of shading indicates a higher value (higher species count, higher Shannon index, or higher evenness).

PEATLAND	SPECIES COUNT	SHANNON INDEX	EVENNESS
VICTORMINE (101)	172	3.3420	0.6833
VICTORMINE (102)	351	3.6686	0.6448
WRPF	193	4.2334	0.8119
VMOE	198	3.3972	0.6451
MER BLEUE	1699	5.1828	0.6994
CARTIER	1093	4.6257	0.6630
MCLEAN	1395	4.9061	0.6791
PURVIS ROAD	1545	4.8471	0.6610
CRANBERRY	1675	5.0721	0.6840
BIG RUN	2046	5.4929	0.7209
LONG LAKE	1804	5.9564	0.7967
DAISY LAKE	1026	5.7118	0.8240
WHITSON LAKE	1449	6.1148	0.8405
PINEOLA	718	5.9867	0.9128
SUGAR	716	5.9592	0.9073
TATER	1593	5.9943	0.8188
RIPSHIN	856	5.8029	0.8826
WRIF	211	4.5808	0.8591
VMOE FEN	1406	5.5566	0.7672
CEDAR	4020	6.6025	0.8267

Table 3 Indicator phyla analysis across all four wetland types at three depths of peat. Phyla are all Bacteria unless designated with a caret (^) for Archaea; a minus symbol (-) indicates absence. Significance codes (p-value): 0 '***' 0.001 '**' 0.01 '*' 0.05.

Indicator Phylum	Bog	Poor Fen	Intermediate Fen	Rich Fen
		Depth to peat = 10 cm		
FBP	0.01% *	-	-	-
WS5	-	-	0.18% **	-
PAUC34f	-	-	-	0.03% **
GOUTA4	-	-	-	1.16% **
GN04	-	-	-	0.94% *
OC31	-	-		0.05% *
GN02	-	-	0.04% ***	
WS3	-	-	0.16% **	
OP11	-	-	0.30% *	
SR1	-	-	0.02% *	
Tenericutes	-	-	0.09% *	
AD3	-	-	0.07% **	
		Depth to peat = 30 cm		
WS5	-	-	0.02% **	-
PAUC34f	-	-	-	0.01% ***
GOUTA4	-	-	-	0.40% ***
LCP.89	-	-	-	0.02% *
TA06	-	-	-	0.01% *
Fusobacteria	-	-	-	0.01% *
Caldiserica	-	-	-	0.01% *
OC31	-	-	-	0.04% *
Gemmatimonadetes	-	-	0.18% **	
OP11	-	-	0.63% ***	
GN04	-	-	0.21% **	
WS3	-	-	0.24% ***	
OP8	-	-	0.28% *	
Tenericutes	-	-	0.03% *	
SR1	-	-	0.02% *	
GN02	-	-	0.03% *	
WPS.2	-	-	0.04% ***	
AD3	-	-	0.77% **	
		Depth to peat = 60 cm		
WPS.2	0.07% *	-	-	-
GOUTA4	-	-	-	0.42% *
TM7	-	-	-	0.02% *
MVS.104	-	-	-	0.02%*
GN04	-	-	1.16% ***	
Gemmatimonadetes	-	-	0.15% ***	
OP11	-	-	0.76% *	
^Parvarchaeota	-	-	1.95% *	
WS3	-	-	0.27% **	
GN02	-	-	0.05% **	

FIGURES

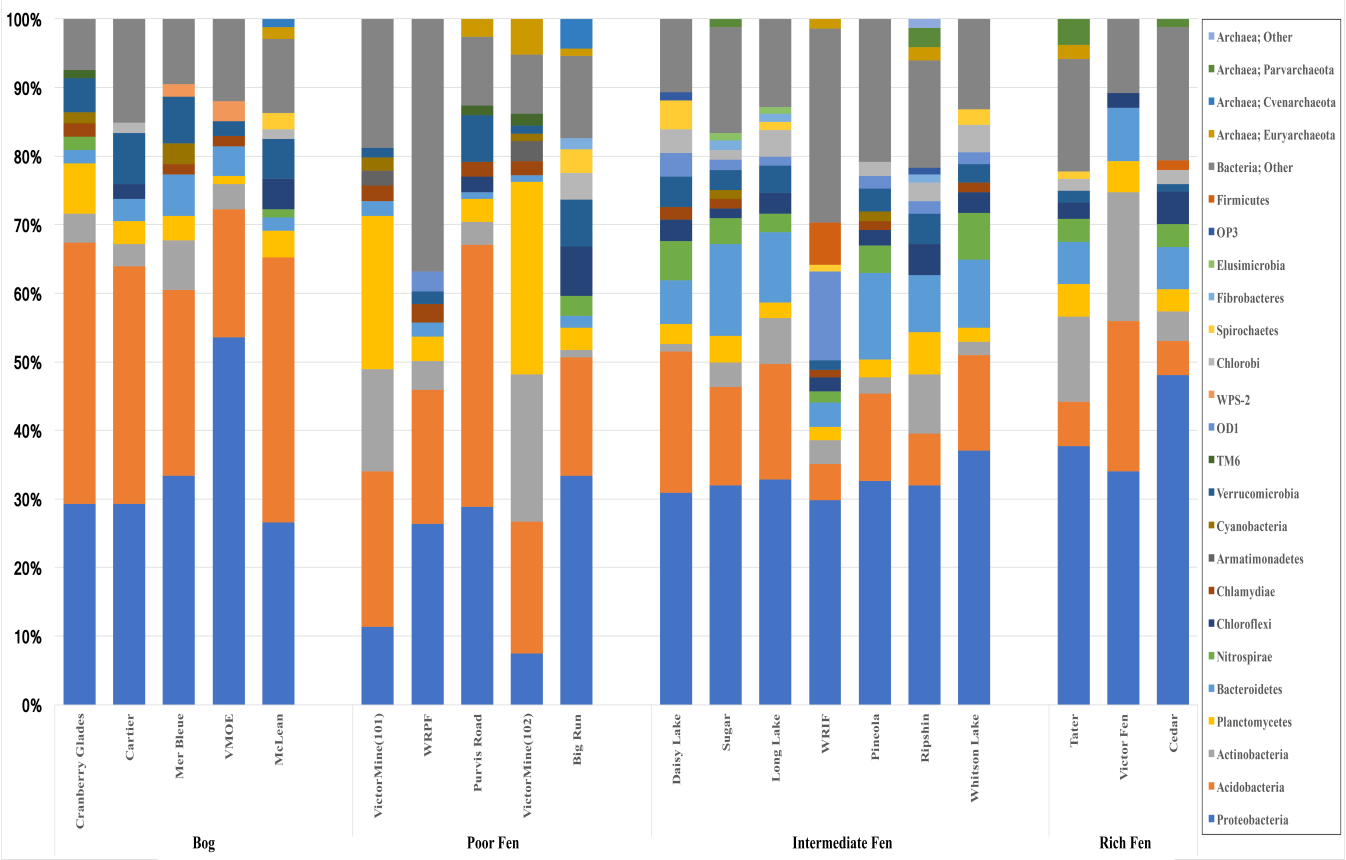
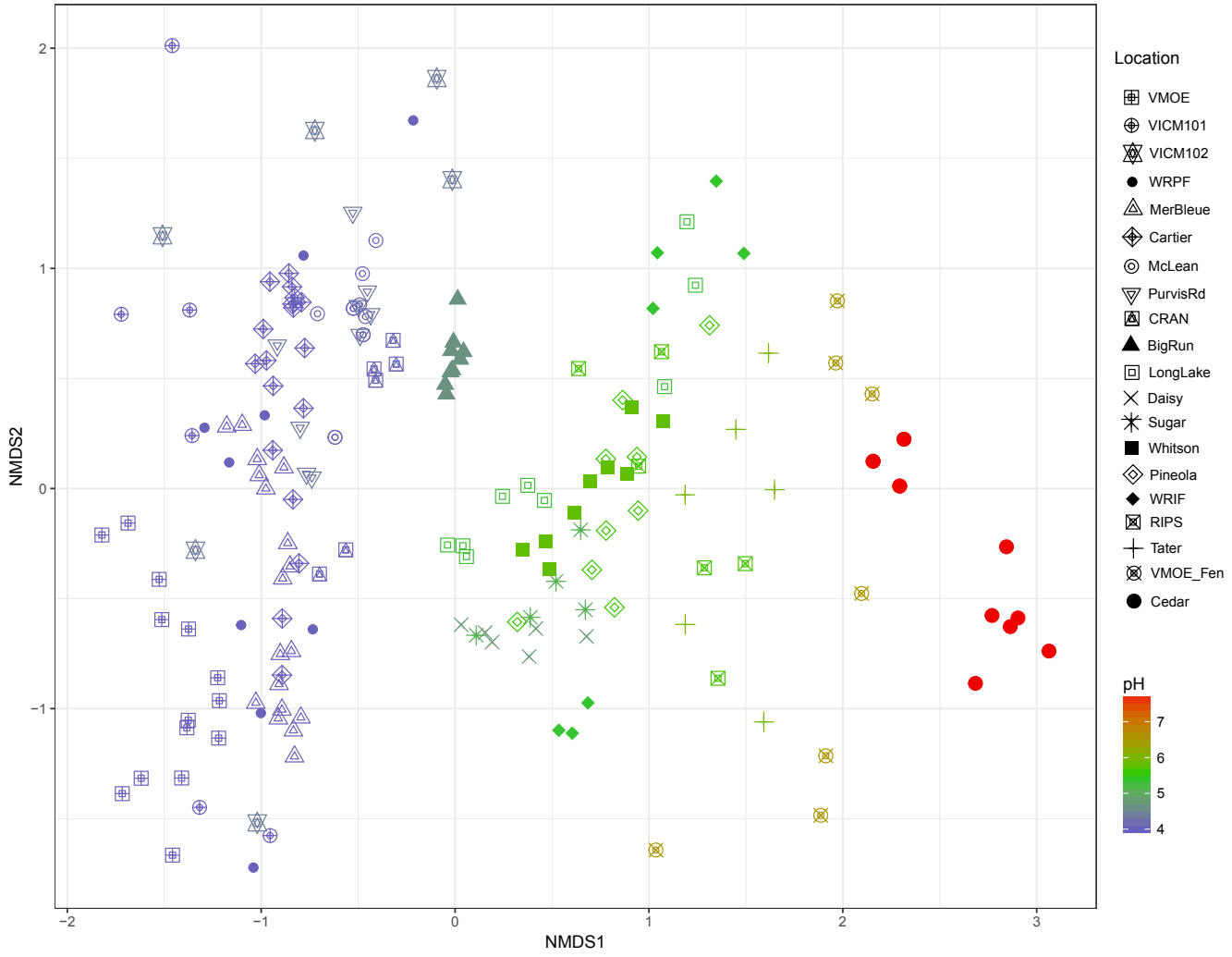


Fig. 1 Taxonomic bar plot with total percentages (all depths averaged) of bacterial and archaeal abundance at phylum classification.

A


NMDS2
NMDS1
2
1
0
−1
−2
−1
0
1
2
3
Location
VMOE
VICM101
VICM102
WRPF
MerBleue
Cartier
McLean
PurvisRd
CRAN
BigRun
LongLake
Daisy
Sugar
Whitson
Pineola
WRIF
RIPS
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VMOE_Fen
Cedar
pH
7
6
5
4

B

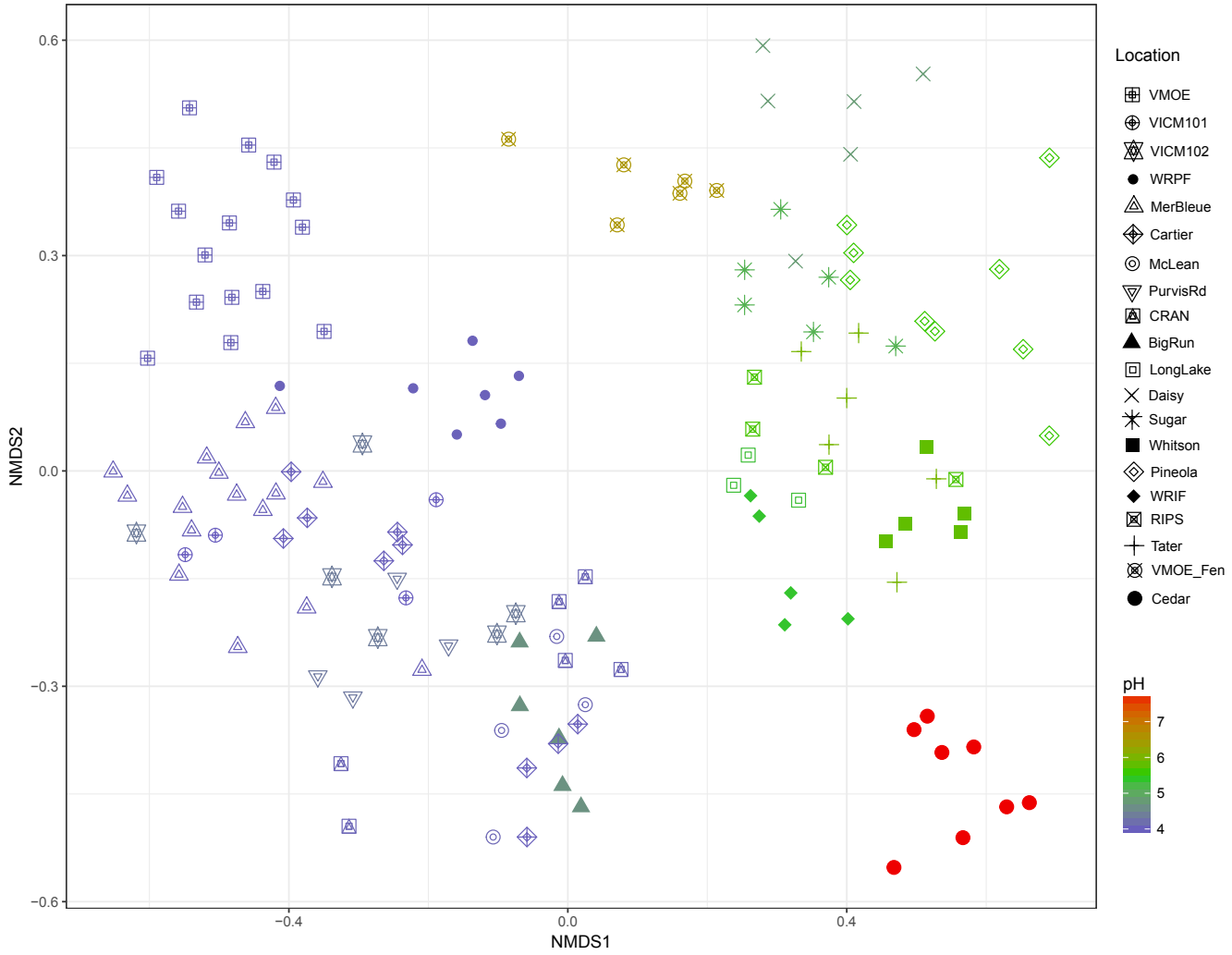


Fig. 2 Non-metric multidimensional scaling (NMDS) plots of (A) bacterial/archaeal communities, and (B) fungal communities. Ordination is based on Bray-Curtis dissimilarity values

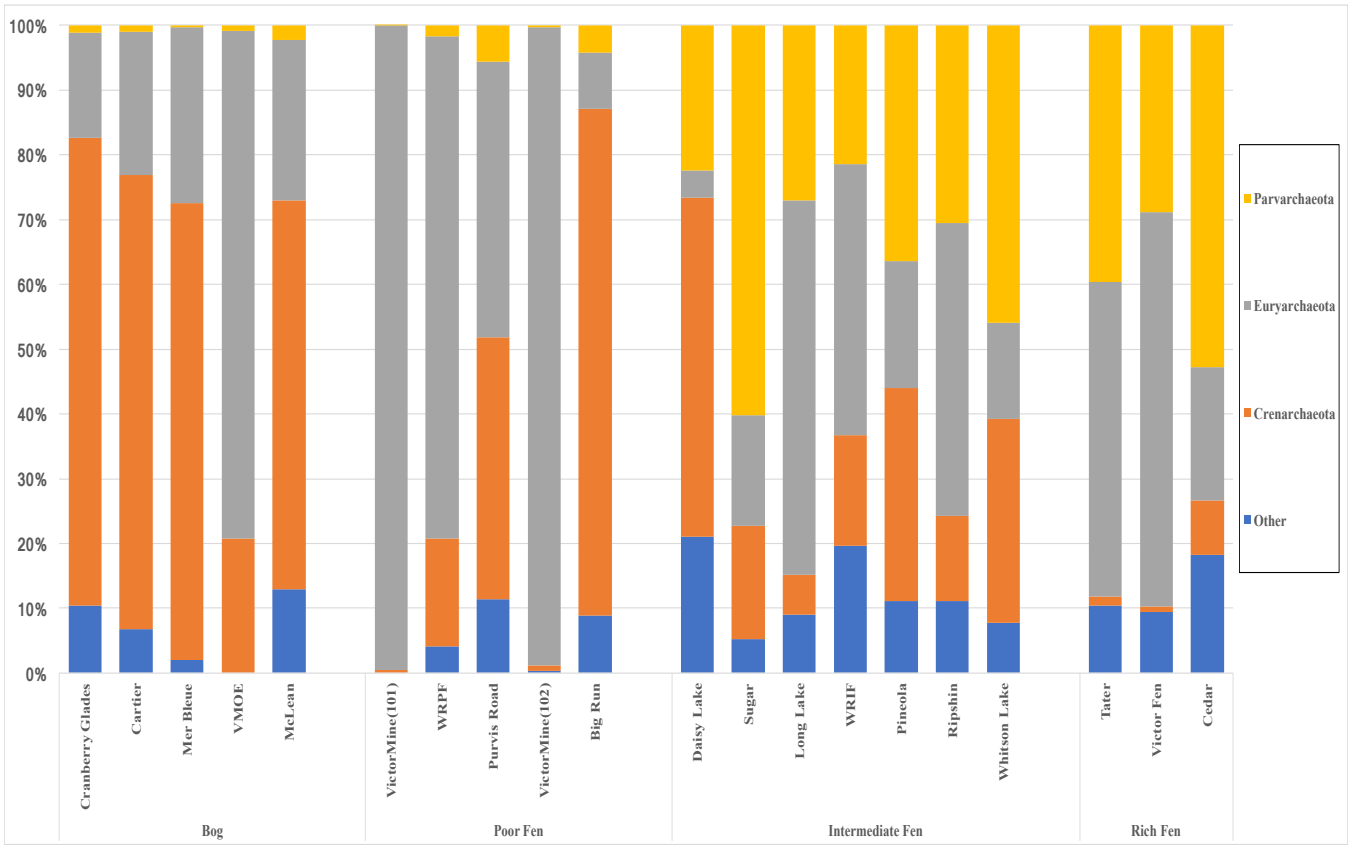
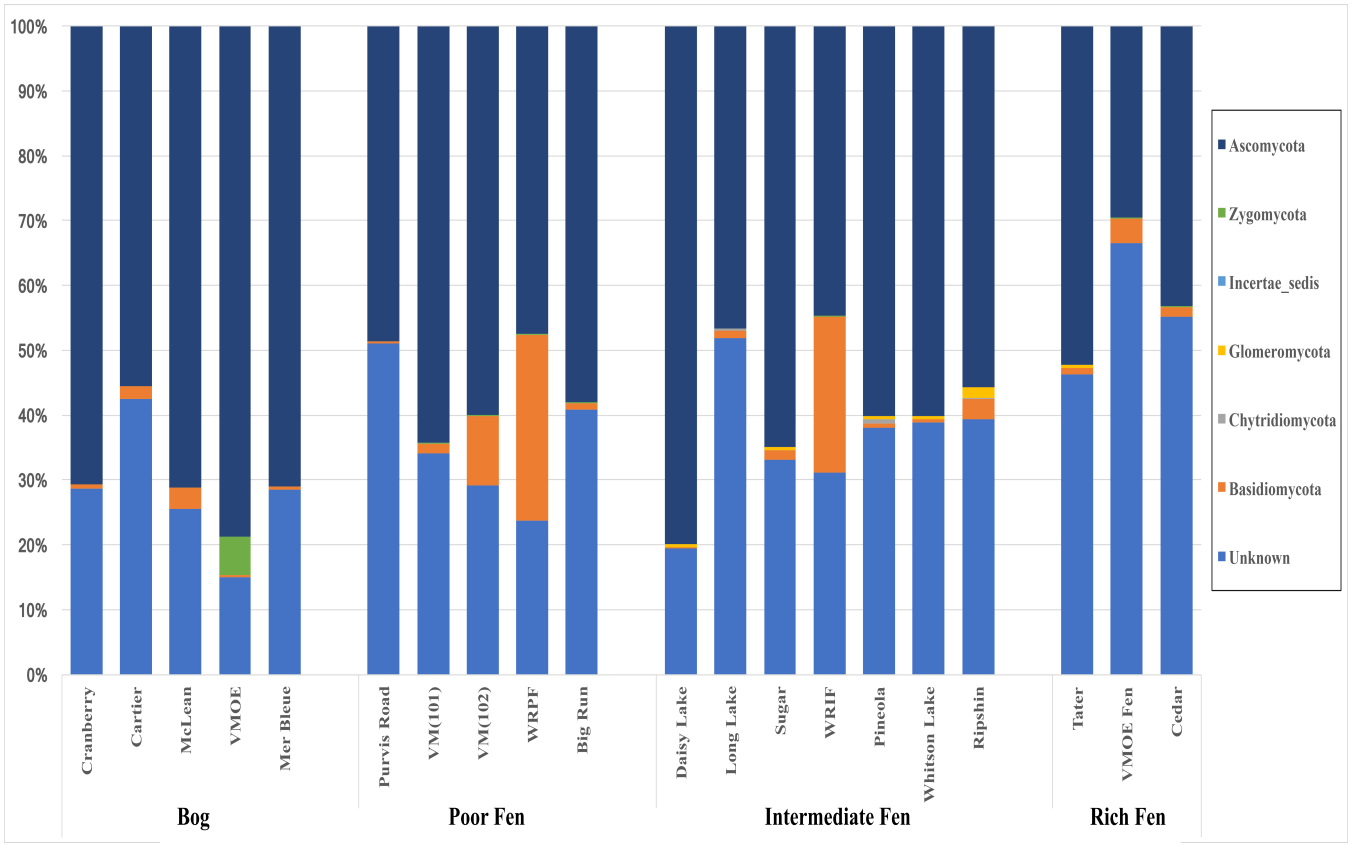


Fig. 3 Phylum classification of archaea per site (all depths averaged).

A


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Ascomycota
Zygomycota
Incertae_sedis
Glomeromycota
Chytridiomycota
Basidiomycota
Unknown
Cranberry
Cartier
McLean
VMOE
Mer Bleue
Purvis Road
VM(101)
VM(102)
WRPF
Big Run
Daisy Lake
Long Lake
Sugar
WRIF
Pincola
Whitson Lake
Ripshin
Tater
VMOE Fen
Cedar
Bog
Poor Fen
Intermediate Fen
Rich Fen

B

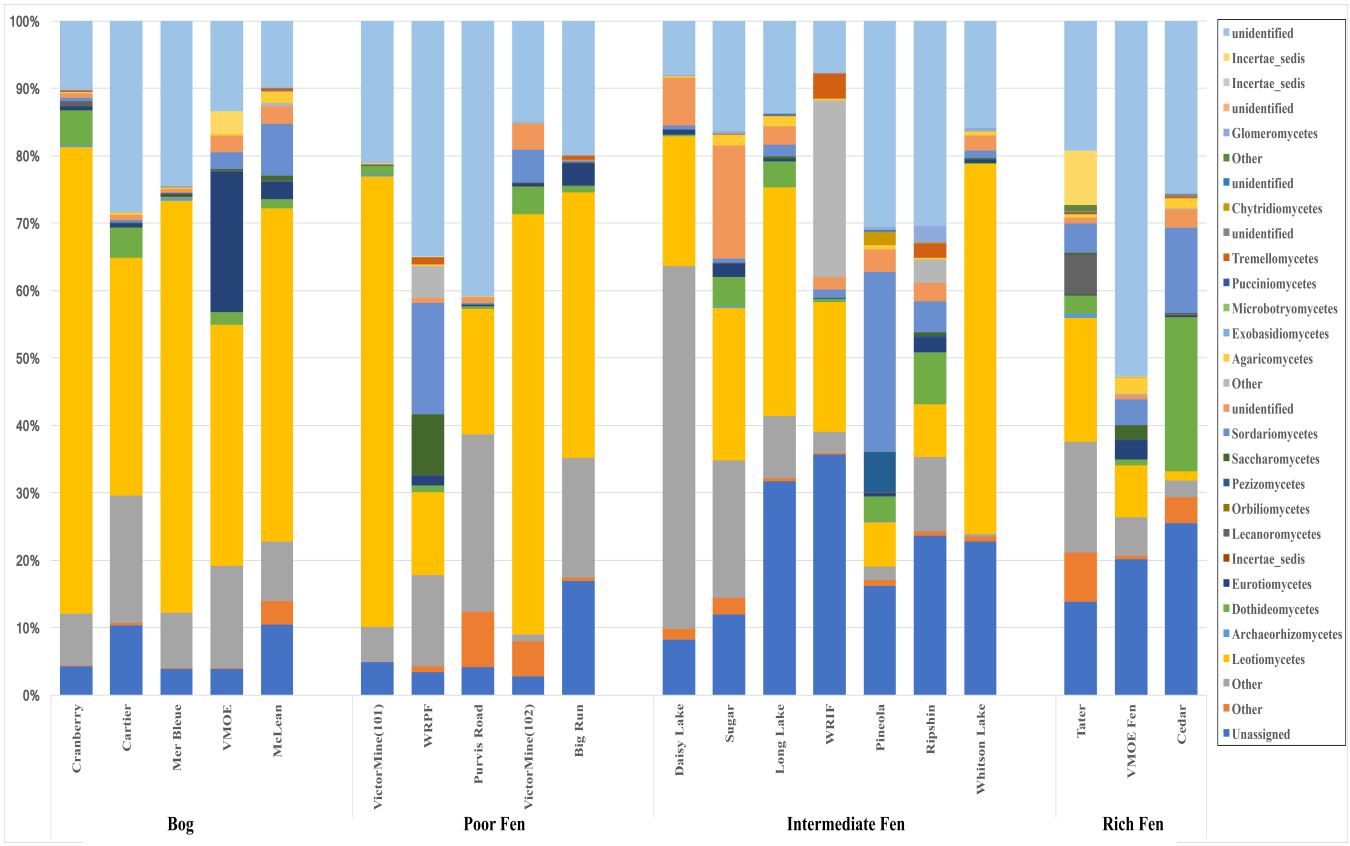


Fig. 4 Taxonomic bar plots with total percentages (all depths averaged) of fungal abundance at the (A) phylum and (B) class level.

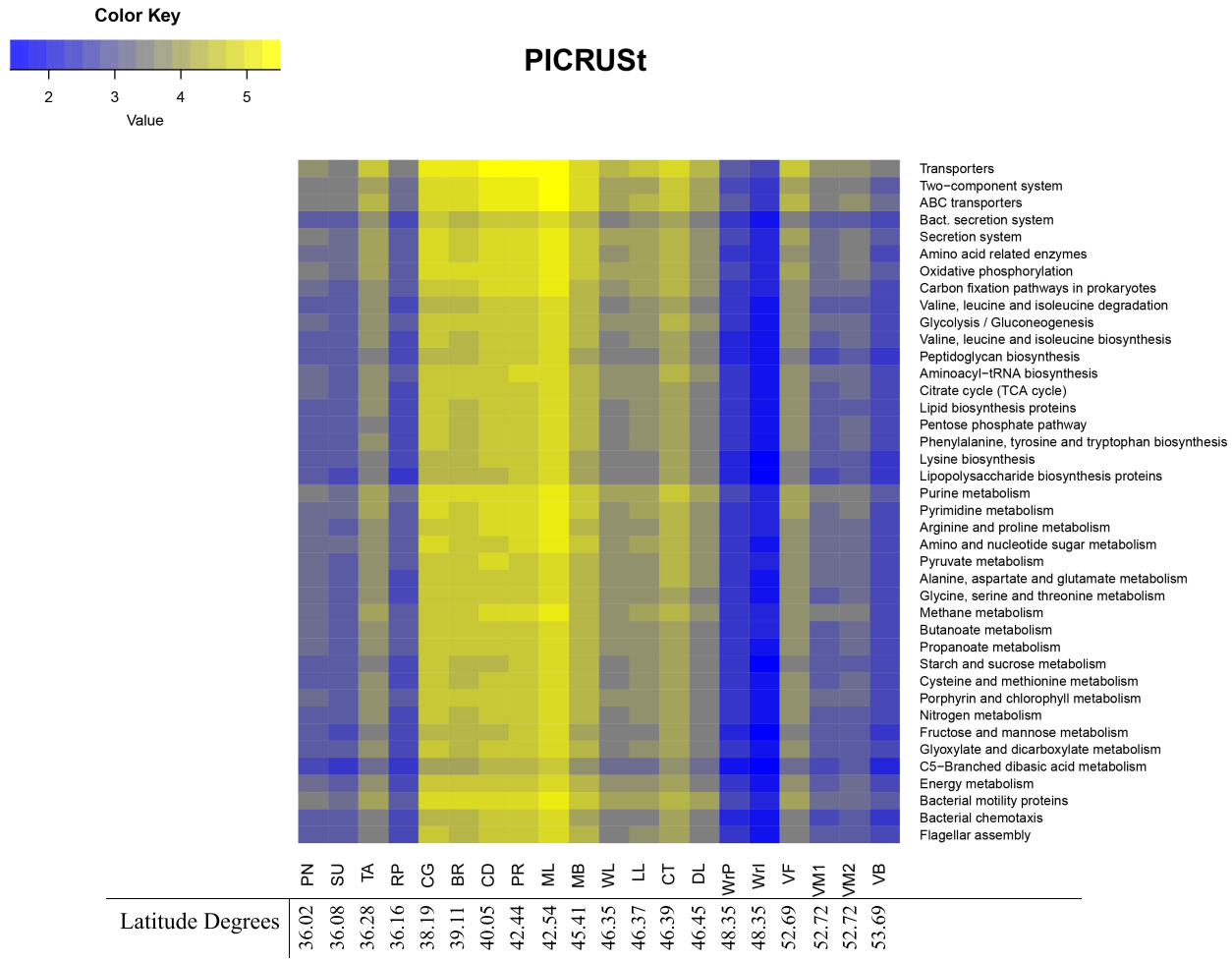


Fig. 5 Heat map of KEGG level three classification for peatlands of interest (generated via PICRUSt predictive genomic software. Peatlands along the x axis are ordered from the most northern latitude to the most southern latitude. Abbreviations include {(Pineola; PN), (Sugar; SU), (Tater Hill; TA), (Ripshin; RA), (Cranberry Glades; CG), (Big Run; BR), (Cedar; CD), (Purvis Road; PR), (McLean; ML), (Mer Bleue; MB), (Whitson Lake; WL), (Long Lake; LL), (Cartier; CT), (Daisy Lake; DL), (White River Poor Fen; WrP), (White River Intermediate Fen; WrI), (Victor Fen; VF), (Victor Mine 101; VM1), (Victor Mine 102; VM2), (Victor Bog; VB).

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SUPPLEMENTAL FIGURES/APPENDIX

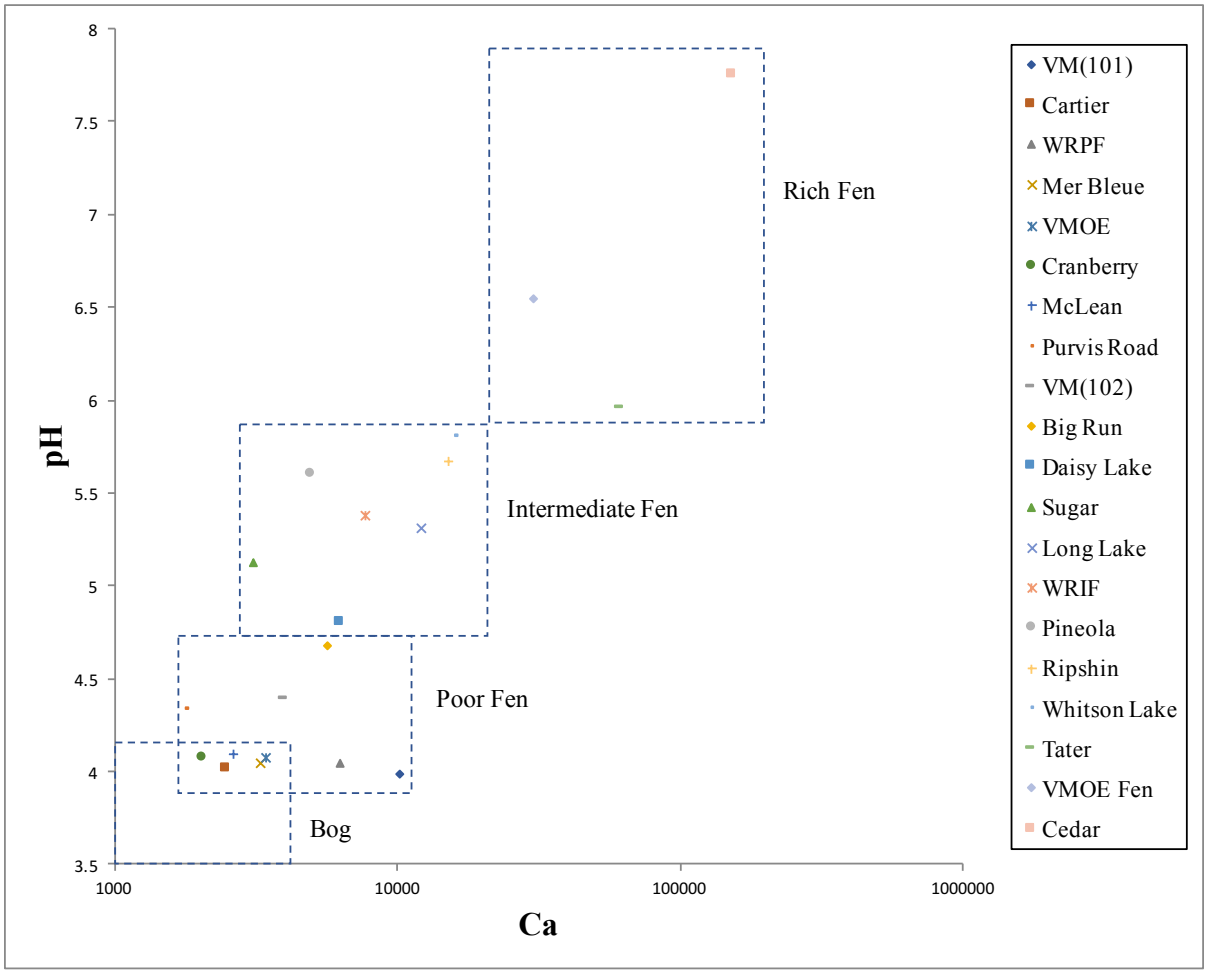

Fig. 1 Peatland classification based on the relationship between pH and calcium (Ca) content.

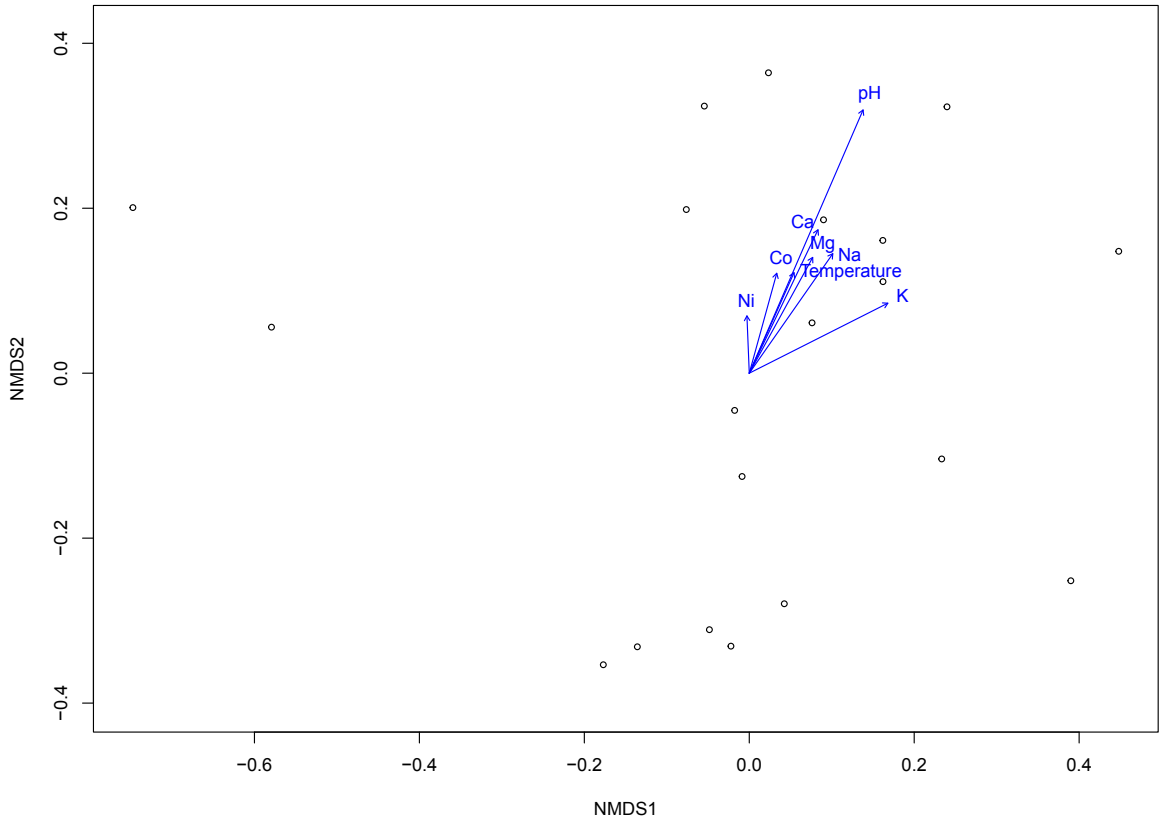

Fig. 2 Non-metric multidimensional scaling (NMDS) biplot of temperature, pH, Ca, Ni, K, Mg, Co, and Na.

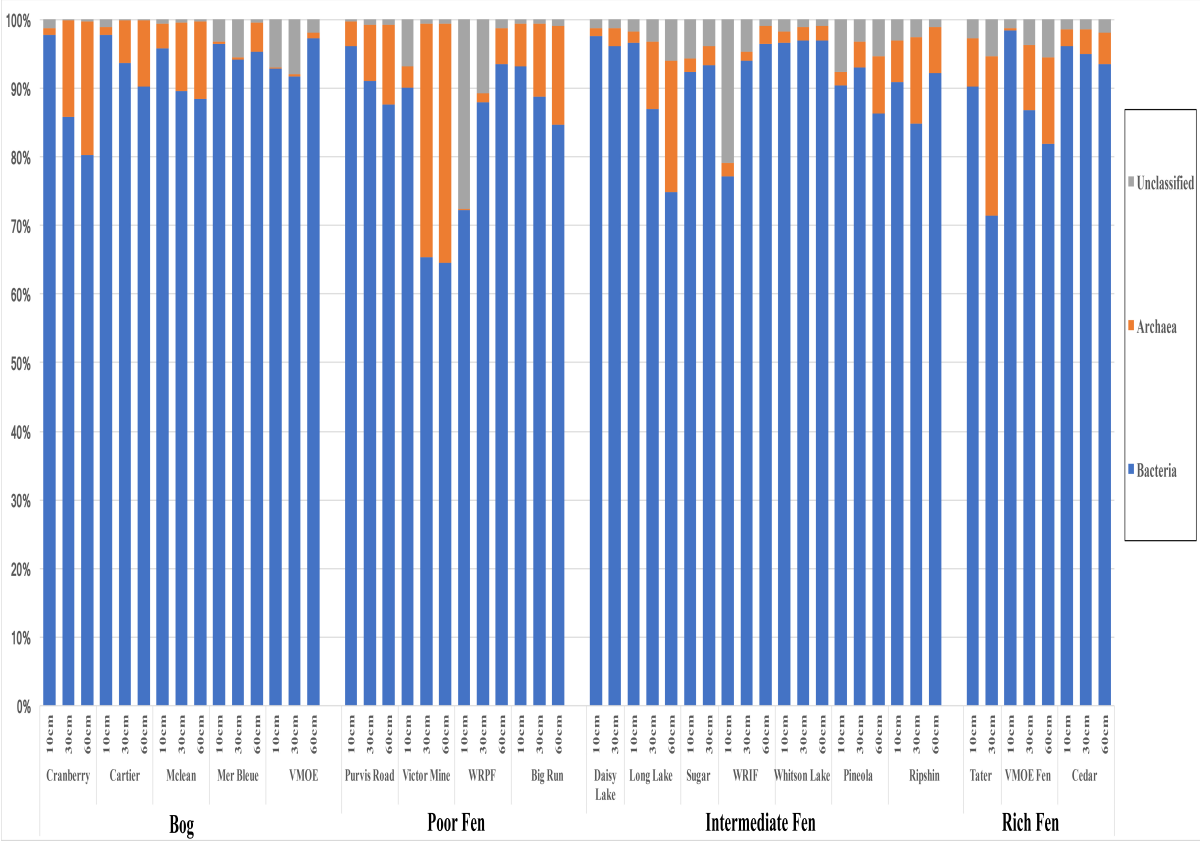

Fig. 3 Taxonomic bar plots showing relative abundance values (%) for domain-level classification of bacterial and archaeal sequences at 10, 30, and 60 cm depths, across 20 peatland areas

VITA

James Donald Seward III was born in Greenville, South Carolina on the 20th of August, 1992. James graduated from Clemson University with a B. S. in Biology and a minor in Microbiology in 2016. During his time at Clemson, James realized his passion for environmental research while working for Dr. Mike Henson. James came to Appalachian State University in 2016 to pursue at M. S. in Cell and Molecular Biology with Dr. Suzanna Bräuer. After completing his degree in August 2018, James will pursue a Ph.D. in Boreal Ecology in the laboratory of Dr. Nathan Basiliko at Laurentian University in Ontario, Canada.